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PROTEINS IN MULTIPLE MYELOMA. VIII. BIOSYNTHESIS OF ABNORMAL PROTEINS*

By

F. W. Putnam† and A. Miyake

Multiple myeloma is a fatal tumor of the plasma cells. It is generally accompanied by hyperglobulinemia, Bence-Jones proteinuria, or both aberrations in protein synthesis. Although the myeloma serum globulin and the unique urinary protein are thought to be formed within the tumor cell, no metabolic relationship has yet been demonstrated between the two types of abnormal proteins. There is evidence that different patients produce individually characteristic globulins that are related to normal serum proteins, yet distinguishable from them, and that the Bence-Jones proteins excreted by different patients are not identical. It is known that the Bence-Jones proteins are not derived by renal cleavage of the abnormal serum globulins, and it has been suggested that the two types of pathologic proteins may be synthesized independently. The urinary protein is formed rapidly from administered amino acids and is excreted rapidly.

In order to investigate whether the synthesis of Bence-Jones protein is related to that f the myeloma globulin, 450 μc of DL-glutamic acid-1-C^{14} were injected into a patient ho was catheterized to permit frequent withdrawal of urine specimens. The subject and protocol of the experiment and the metabolic fate of the racemic amino acid are given in the accompanying paper (p. 35).

Significant radioactivity was not detected in either the Bence-Jones protein or the myeloma globulin until about 40 minutes after injection.

Specific activity rate curves were obtained for four nonprotein metabolic products of the injected amino acid, namely 1) expiratory CO_2, 2) the urinary urea CO_2 liberated by urease, 3) the CO_2 released by ninhydrin from urinary amino acids, chiefly D-glutamic acid, and 4) the CO_2 liberated from L-glutamic acid of serum by the optically specific glutamic acid decarboxylase from E. coli (Table 1). Rate data were also obtained for two fractions of the Bence-Jones protein that differed in solubility and for the myeloma serum globulin prepared by salt precipitation.

By chemical analysis the total serum protein was found to be 8.1%, with an albumin-globulin ratio of 0.7. Electrophoretic analysis by the Tiselius method revealed a sharp peak in the γ-globulin region characteristic of myeloma globulin. This component had a mobility of 1.5×10^{-5} cm^2 sec^{-1} $volt^{-1}$ and comprised 30% of the total serum protein. The

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*Summary of a paper that appears in The Journal of Biological Chemistry, 231:671 (1958).

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THE METABOLISM OF DL-GLUTAMIC ACID-1-C^{14} IN MAN*†

By

F. W. Putnam,‡ A. Miyake, and F. Meyer

Despite its alleged occurrence in human and animal tumors, D-glutamic acid has not been found to be utilized to any appreciable extent in experimental animals. The fate of D-glutamic acid in man, however, has not been fully investigated. In the course of study of the biosynthesis of abnormal proteins in multiple myeloma, DL-glutamic acid-1-C^{14} was injected intravenously into a patient. Frequent blood and urine samples were taken, and the expiratory CO_2 was collected at intervals. Time curves were determined for the radioactivity of 1) expiratory CO_2, 2) urinary urea, 3) urinary amino acids, 4) serum glutamic acid, 5) urinary Bence-Jones protein, and 6) serum γ-globulin (the myeloma globulin). A comparison was also made of the radioactivity of the CO_2 released by the reaction of urinary amino acids and of the hydrolyzed Bence-Jones protein with ninhydrin versus glutamic acid decarboxylase. The L-glutamic acid was metabolized rapidly. However, it was excreted apparently unchanged in the urine and was not incorporated significantly into the Bence-Jones protein, which is believed to originate in the tumor cells.

DL-glutamic acid-1-C^{14}, with an activity of 10 μc per mg, was chosen for this study because the radioactivity is completely liberated from the L form on incubation with glutamic acid decarboxylase. This not only permits a differentiation of the L and D isomers but also affords a direct method for measuring the specific activity of glutamic acid in protein hydrolysates without isolation of the amino acid. Prior to administration, the glutamic acid (10 μc per mg) was dissolved in a 0.9 per cent NaCl-0.5 per cent glucose solution, filtered through ultrafine sintered glass, and tested for toxicity and sterility. No carrier glutamic acid was administered.

The purified, lyophilized, salt-free protein samples were counted on planchets; the specific activity corrected for self-absorption is expressed as cpm per mg of protein. Protein samples were hydrolyzed by autoclaving for 12 hours with 6 N HCl. The hydrolysates were concentrated in a flash evaporator with repeated washings to remove the acid. The amino acids in the hydrolysates were then decarboxylated by reaction with ninhydrin at 100° or by incubation at 37° with successive aliquots of glutamic acid decarboxylase. The amino acids in urine samples of 0.3 or 3.0 ml were likewise decarboxylated with nin-

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†Summary of a paper that appears in The Journal of Biological Chemistry, 231:657, '8.

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hydrin but in the presence of 15 mg of L-glutamic acid as carrier. Enz matic decarboxylation of 1.0 ml serum samples was also carried out in the presence of 1 mg of carrier L-glutamic acid. In all the decarboxylation reactions the system was firs flushed with nitrogen to remove preexisting CO_2; the CO_2 released by the reaction was t apped with $Ba(OH)_2$ and counted as $BaCO_3$ in aluminum planchets with a surface of 3.47 cm The activities are given as cpm for infinitely thick samples. The specific activity of expir tory CO_2 (expressed as millimicrocuries per millimole of CO_2) was measured by n eans of a vibrating reed electrometer. The specific activity of urinary urea CO_2 was also etermined.

Serum protein fractions were prepared either by starch zone elect ophoresis or by salt precipitation. The Bence-Jones protein was isolated by ammonium sulfate precipitation and prolonged dialysis against distilled water. These protein prepa ations were characterized by electrophoresis in the Tiselius apparatus and by sediment tion in the analytical ultracentrifuge.

The patient (H. H.), a 67 year-old male, was admitted to the Argon e Cancer Research Hospital for treatment and terminal care. For therapy, urethane was g en throughout the experiment and radiation just prior to it. Laboratory study revealed Be ice-Jones proteinuria, hyperglobulinemia, moderate anemia (12 g per cent of hemoglobir , and severely depressed renal function. Blood urea nitrogen rose progressively from 2: mg per cent to 176 mg per cent, and the urea clearance fell terminally to a value of 2. , as calculated by the square root method. The diagnosis of multiple myeloma was confir: ed by postmortem examination. Kidney damage was extensive and typical of the disease.

Approximately 450 μc of DL-glutamic acid-1-C^{14} were given intra enously over a 15-minute interval to the fasting patient. In the first 24 hours, 19 blood samples were obtained by intravenous catheter, the first at 15 minutes after the start o: the injection. A complete urine collection was maintained with 24 samples being taken l urethral catheter on the 1st day. The catheters were then removed, and blood samples w e discontinued; but complete urine collection was kept up for a week with a total of 49 s ecimens. Blood was allowed to clot, and the serum specimens were frozen for later stu y. Aliquots of the urine were taken for immediate isolation of the Bence-Jones protein, a d the balance was frozen for storage. The expiratory air was sampled at intervals for the first 8 hours. Except for an initial discomfort, the experiment had no adverse effects. N trogen intake studies were carried for 2 months following the injection.

In view of the known metabolic pathways of glutamic acid, a sharp arly maximum in the specific activity of expiratory $C^{14}O_2$ was expected. This maximum ccurred at about 25 minutes and was followed by an exponential decline in radioactivity with a half time of 85 minutes (Figure 1). Three and one-tenth per cent of the injected acti ity or 6.2 per cent of the activity in the L form was exhaled in the first 8 hours. In the 5-n nute period at the time of the maximal specific activity of expiratory CO_2, 0.72 μc of C^{14} $)_2$ was exhaled with only 0.02 μc in the 5-minute period at 8 hours after injection. At 2 hours, the activity of expiratory CO_2 would have been negligible.

The curves giving the rate of urinary excretion of C^{14}-amino acid: shown in Figure

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RADIOCARBON AND MEDICAL RESEARCH

By

G. V. LeRoy

Exploration of metabolic processes in man, and in laboratory and domestic animals, is surely one of the most important of the peaceful uses of nuclear energy. The elegant studies of Schoenheimer and his students revolutionized biochemistry, and his concept of the dynamic state of the body's constituents may be considered as a quantum jump forward in our understanding of health and disease. We are fortunate in having at our disposal, isotopes—stable and radioactive—of all of the elements involved in living organisms. Of these isotopes, carbon-14 is the most useful for studies of metabolism. Its beta radiation can be measured easily and accurately; and the low energy of the radiation allows one to perform reliable tracer studies with insignificant radiation exposure. Finally, virtually every substance of interest to a biochemist—metabolically speaking—can be obtained labeled with radiocarbon. Broadly speaking, four types of biologic research in which C^{14} is used are in progress: photosynthesis, enzymatic reactions in noncellular systems, biochemical reactions in cell-containing systems, and metabolic studies in intact organisms.

From this research has evolved an elaborate scheme of the movement of carbon in e biosphere, beginning with the carbon dioxide used in photosynthesis; and ending with the carbon dioxide exhaled with each breath by animals. It is literally true that the conversion of solar energy to the energy used for human activity can be described in terms of the natural history of carbon dioxide, and can be studied, step-by-step, with the help of radiocarbon.

There are formidable problems to be solved in the development of methods for the use of radiocarbon for metabolic studies in man. Consider, for example, an investigation of the metabolism of glucose, a sugar containing 6 carbon atoms. It is possible to obtain uniformly-labeled glucose produced biosynthetically, and it is also possible to obtain glucose labeled in the 1, 2, or 6 position. When labeled glucose is given to a man (or an animal—or a cellular system—or a subcellular system), the majority of it is oxidized promptly to yield carbon dioxide, and the remainder moves along various pathways, eventually to

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Excerpts from a lecture delivered, October 13, 1958, at a symposium on Nuclear Energy Uses for Pharmaceutical and Organic Chemists, sponsored by the Nuclear Science and Engineering Corporation.

The work described in this report was carried on as a team project. The key members of the team were George T. Okita, Edward C. Tocus, and Donald Charleston of the staff of the Argonne Cancer Research Hospital, USAEC. Valuable assistance was rendered by Paul Meier, Assistant Professor of Statistics, and by the following students, medical d graduate: Philip Gradolph, Burton Rodin, George Kirby, William Gross, and Robert rnard, The University of Chicago.

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be eliminated as carbon dioxide and urea. More specifically, we can say that the tracer dose of glucose enters the glucose pool. This pool receives glucose from the food, from the breakdown of glycogen, and from the process of gluconeogenesis. From the pool, glucose is withdrawn by cells to be oxidized as a source of energy, to be condensed into the storage substance, glycogen, and to be used in the synthesis of other organic compounds. We assume that the concentration of radio-glucose in the blood is representative of its concentration in the pool, and obtain repeated samples of blood for radiochemical analysis. These analyses demonstrate the die-away of glucose specific activity (SA) as a function of time and permit us to estimate values for the size and rate of turnover of the glucose pool. Ordinarily, 8 to 10 samples are needed to construct a reliable die-away curve for a substance that moves as rapidly as glucose. Each sample must be processed to obtain radiochemically-pure glucose for determination of SA. So far, all we have learned is the rate of dilution of the labeled material in the glucose pool. The behavior of the pool depends upon at least six separate reactions (see above), some of which (e.g., glycogen-formation-degradation) cannot be studied except by analysis of tissues, and all of which are probably steady-state reactions. Of all the six reactions, only oxidation yields a product that can be analyzed conveniently.

As a glucose molecule is oxidized, the carbon dioxide formed enters the body's carbon dioxide pool. This pool turns over rapidly, and some fraction of the CO_2 entering it is eliminated in expired air. The carbon dioxide pool is in stable equilibrium with the carbonate component of the bones, and some fraction of carbon dioxide flowing through the pool is utilized by cells for the synthesis of organic compounds (e.g., glucose, uric acid, etc.). The changing SA of the carbon dioxide pool can be observed by studying expired air since the specific activity of CO_2 is identical in blood and expired air. The kinetics of the carbon dioxide pool can be measured, using $NaHC^{14}O_3$ as a tracer if we know the volume of CO_2 exhaled per unit time and the die-away of specific activity as a function of time. Most investigators working with expired air collect samples in balloons, absorb the CO_2 in barium or sodium hydroxide, process each sample separately, and assume a steady volume of ventilation for the experimental period. With a large staff of technicians it is possible to perform 2 or 3 experiments a week, but even this schedule is difficult to maintain. After the blood and air samples are analyzed, a complex mathematical treatment, involving several assumptions, is necessary to connect the two sets of data, and to derive a quantitative expression for the rate of utilization—or oxidation—of glucose.

Since it is obvious that C^{14}-labeled glucose can yield valuable information about glucose metabolism in man; some sort of automation seemed necessary if a procedure was to be developed for routine use. To simplify the problem, we decided to study only the expired air, thus eliminating the blood analysis. This precluded an estimate of the size of the glucose pool, but did not prevent an estimate of the relative rate of oxidation of the various portions of the glucose molecule, or of the entire molecule under varied circumstances.

The system we devised consists of a meter to record continuously the volume of ven-

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tilation—the liters of air expired per minute. After the expired air volume is measured, a sample of about one liter per minute is pumped through a trap to remove water vapor, and then through a specially-designed G.M. counter where the C^{14} radioactivity is measured continuously. Another, smaller, sample is pumped through an infrared absorption meter to record continuously the percentage of carbon dioxide. A ratio analyzer computes the C^{14}/C^{12} ratio. All the data are printed on a Speedomax recorder, using 4 of the 6 available channels (Figure 1). The electrical output of the ventilation meter, and of the

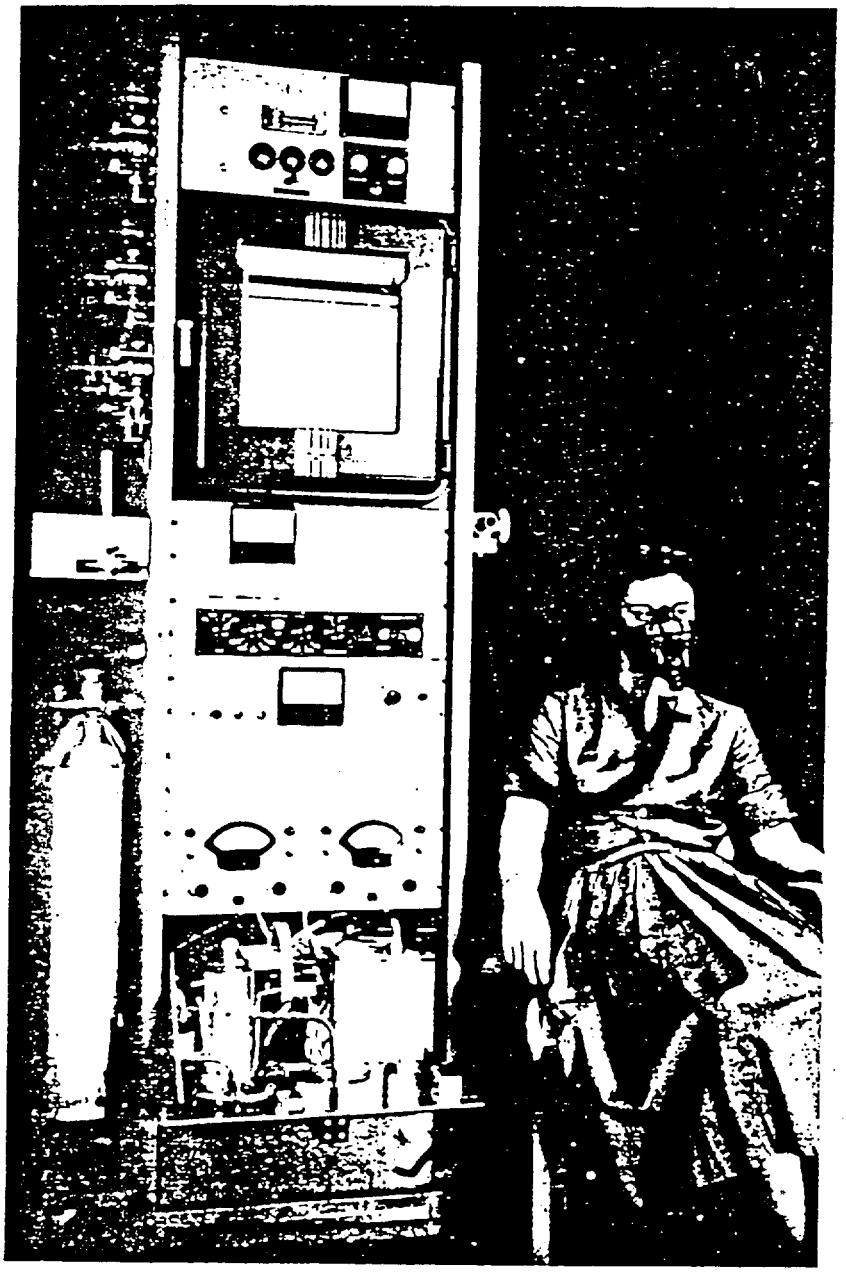

Figure 1. The continuous $C^{14}O_2$ monitor in operation. The open panel at the bottom of the apparatus reveals the 4 G.M. counter and the ventilation meter.

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Geiger counter, consists of pulses that activate a rate-meter. In addition, the pulses produced are totaled in a scaler unit. Thus, we have the following data recorded continuously: rate of ventilation per minute, cumulative volume of ventilation, per cent $C^{12}O_2$, radioactivity of expired air, cumulative excretion of radioactivity, and the specific activity of carbon dioxide.*

To study the metabolic fate of labeled glucose, we first estimate the size and the turnover of the carbon dioxide pool. This involves the intravenous injection of a single dose of C^{14}-labeled sodium bicarbonate. For an average man, 5 μc is adequate. The apparatus is run for 60 to 70 minutes after the tracer dose is given. By the end of this time, the counting rate in expired air has decreased to 2 to 3 times background. The counter is 50 per cent efficient, and can measure as little as 5×10^{-5} μc/liter of air accurately. The data for the bicarbonate experiment are transferred to IBM cards, and a program and the data are fed into a 650 computer. Within a few minutes, we have a print-out (Figure 2) of the volume of carbon dioxide per minute, two estimates of pool size, and two estimates of turnover rate.†

On succeeding days, the subject is given tracer doses of glucose labeled in various ways. We assume that labeled carbon dioxide resulting from oxidation of the tracer enters the bicarbonate pool at an exponential rate. Once in the pool, it behaves in the same fashion as the carbon dioxide from the sodium bicarbonate; that is, a certain fraction is exhaled, and the remainder exchanges with bone carbonate and is utilized in biosynthetic reactions. Knowing the parameters of the carbon dioxide pool, we can calculate the value of the exponent which describes the rate of oxidation of the labeled glucose. We also, of course, can measure readily the fraction of the tracer dose oxidized at various intervals of time after administration. The computations employed to analyze the glucose curves are still done by hand. Within a few months they will be programmed for machine analysis. When the two programs have been thoroughly checked out, a converter will be installed to convert the data recorded on the Speedomax to the form required for machine analysis. With this step, our apparatus will be completely automatic except for calibration tests and we will be able to study several patients per day.

The concept on which this work is based may be stated briefly, as follows:

- Substances (e.g., glucose) containing more than one carbon atom are broken down by the body into simpler radicals or compounds.
- These basic units are utilized in different ways.
- The difference depends upon the enzymatic pathways that are in operation.
- The difference is reflected in the rate at which labeled carbon dioxide appears in expired air.

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*A technical report describing the instrument is in preparation.

†A paper describing the analyses of $C^{14}O_2$ die-away curve is in preparation.

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"ATOMS FOR PEACE" UNITED STATES COMPUTER FACILITY 2nd INTERNATIONAL CONFERENCE GENEVA 1958

"LES ATOMES POUR LA PAIX" HALLE DES SYSTÈMES À CALCULER DES ÉTATS UNIS 2me CONFÉRENCE INTERNATIONALE GENÈVE 1958

«АТОМЫ ДЛЯ МИРА» ПОМЕЩЕНИЕ ДЛЯ РАСЧЕТНЫХ МАШИН СОЕДИНЕННЫХ ШТАТОВ 2-е ИНТЕРНАЦИОНАЛЬНОЕ СОВЕЩАНИЕ ЖЕНЕВА 1958

"ÁTOMOS PARA LA PAZ" SERVICIO COMPUTADOR DE LOS ESTADOS UNIDOS 2a CONFERENCIA INTERNACIONAL GINEBRA 1958

KINETIC MEASUREMENT OF METABOLISM OF C-14 LABELED INTERMEDIATES
IN NORMAL AND PATHOLOGICAL PATIENTS
FROM THE SCHOOL OF MEDICINE UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS, U.S.A.

SUMMARY OF RESULTS FROM SODIUM BICARBONATE C-14

1	MICROCURIES OF SODIUM BICARBONATE C-14 INJECTED	.7000 1
2.	DURATION OF EXPERIMENT (MINUTES)	.6400 2
3	MICROCURIES RECOVERED DURING EXPERIMENTAL TIME	.2243 1
4	PERCENT RECOVERED DURING EXPERIMENTAL TIME	.3204 2
5	MICROCURIES RECOVERED EXTRAPOLATED TO INFINITE TIME	.2496 1
6	PERCENT RECOVERED EXTRAPOLATED TO INFINITE TIME	.3566 2
7	FLOW RATE OF EXPIRATORY CARBON DIOXIDE (MOLES/HOUR)	.6732 0
8	CALCULATED FLOW RATE OF MISCIBLE BICARBONATE POOL (MOLES/HOUR)	.1887 1
9	CALCULATIONS USING STEWART-HAMILTON METHOD	
	A MEAN TIME (MINUTES)	.2692 2
	B MEAN TIME (NEGLECTING THE FIRST TEN MINUTES)	.3011 2
	C HALF TIME (MINUTES)	.2091 2
	D BICARBONATE POOL SIZE (MOLES)	.9475 0
10	CALCULATIONS USING A FITTED EXPONENTIAL METHOD	
	A THE Y INTERCEPT	.2405 3
	B SLOPE OF THE EXPONENTIAL	.3588-1
	C HALF TIME (MINUTES)	.1931 2
	D BICARBONATE POOL SIZE (MOLES)	.8734 0
	E TURNOVER TIME (MINUTES)	.2780 2
	F TURNOVER RATE OF THE BICARBONATE POOL (MOLES/HOUR)	.1884 1

Figure 2. Printout of the analysis of the die-away curve for SA of $C^{14}O_2$ in expired air after a tracer dose of $NaHC^{14}O_3$. This format was developed for the Geneva Conference. The single digit to the right of the tabular data serves to index the position of the decimal point, thus: 0 = .1234; 1 = 1.234; 2 = 12.34, etc.

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Diseases altering specific enzyme systems, or congenital dificiencies of enzyme systems should be manifested by alterations in the rate of elimination of labeled carbon dioxide under particular circumstances.

Certainly, from studies of this sort, we are justified in anticipating a good yield of information. Already we have obtained new information on glucose metabolism in man and in experimental animals. According to current biochemical theory, the principal route for the oxidation of glucose is the Embden-Meyerhof pathway. In recent years, an alternative scheme of glucose catabolism has been described; called variously the hexose monophosphate shunt, the glucose oxidative pathway, or the pentose phosphate pathway. Particular interest in this "new" pathway results from the demonstration that neoplastic cells seem to utilize it to a much greater extent than the Embden-Meyerhof route. Until recently, all studies of these two pathways were carried out using isolated cells, tissue slices, breis, and the like. The operation of the pentose phosphate pathway can be inferred by the rapid oxidation of the 1-carbon of glucose, as compared, say, to the 6-carbon. Similarly, non-function of this pathway should result in comparable rates of oxidation of carbons 6 and 1.

In healthy subjects, the specific activity-time curves for $C^{14}O_2$ after the administration of labeled glucose are as shown in Figure 3. It is apparent that oxidation of carbon-1, proceeds much more rapidly than oxidation of carbon-6. This is strong evidence that the pentose phosphate pathway is active in healthy individuals, and was an unexpected finding. We were fortunate in being able to study two healthy subjects whose red cells (and pre-

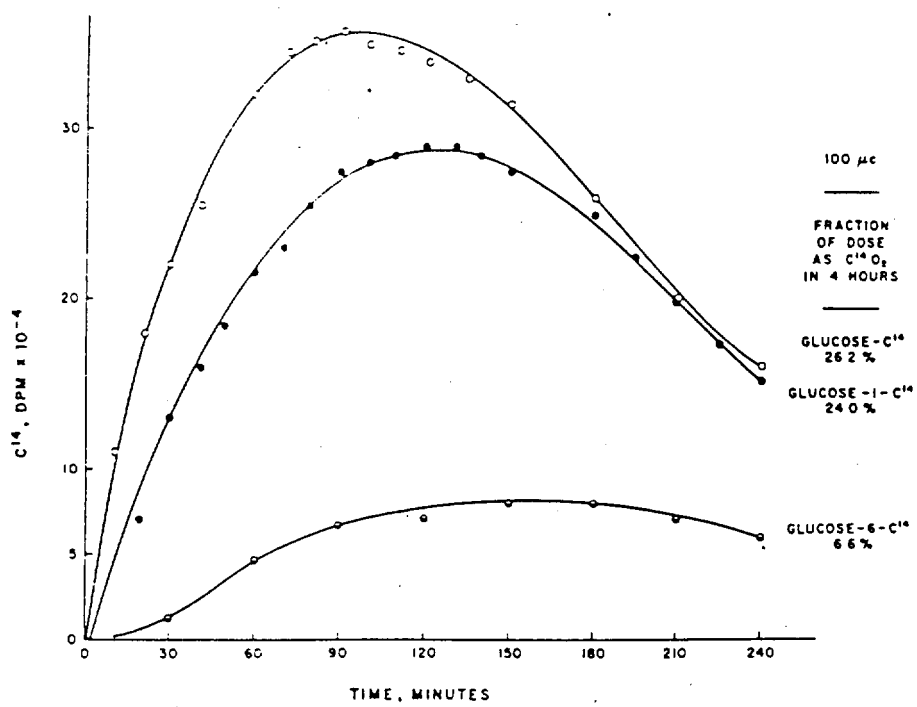

Figure 3. Specific activity-time curves for $C^{14}O_2$ after intravenous doses of glucose labeled in various ways with carbon-14. The subject was a healthy female volunteer.

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STUDIES OF THE METABOLISM OF PROGESTERONE-4-C^{14} IN CHORIOCARCINOMA

By

P. M. Ejarque, E. J. Plotz, C. I. Lupu, R. H. Caplan, and M. E. Davis

In contrast to numerous investigations and extensive knowledge of the production of gonadotrophin by tumors of trophoblastic origin, there is only little information concerning the metabolism and excretion of estrogens and progesterone in patients with choriocarcinoma. The principal metabolite of progesterone, pregnanediol, is usually found in relatively small amounts in the urine of these patients, corresponding with the amounts recovered from the urine during early pregnancy.[1,2] In some cases, pregnanediol may be even absent from the urine;[3] in others, only minimal amounts can be detected.[4,5] On the other hand, Kaufmann and Zander[6] reported a relatively high concentration of the hormone itself in peripheral blood, in lutein cysts, and tumor tissue, but the urinary excretion of pregnanediol was minimal in their two patients.

In connection with our efforts to investigate the metabolism of progesterone in normal and abnormal pregnancy, we decided to study the possibility that there might be an abnormal pathway of the hormone in patients with embryonic tumors.

METHODS AND RESULTS

Progesterone-4-C^{14} that was used was purified by paper chromatography and several recrystallizations. The specific activity of the purified progesterone was 5.15 μc/mg. Two patients with choriocarcinoma* received a single intramuscular injection of the tagged hormone. The actual doses deposited in the gluteal muscle of the patients were 5.27 μc (Patient B.R.) and 37.81 μc (Patient M.E.).

The diagnosis of choriocarcinoma was based on unequivocal histologic evidence. Metastases of the tumor were demonstrated in both patients. They excreted more than 1 million M.U. of chorionic gonadotrophin in a 24-hour urine specimen.

Complete 24-hour urine specimens were collected for periods of 6 days after the administration of the radioactive hormone. An aliquot of the daily urine collection was combusted to carbon dioxide in a vacuum combustion line. The radioactivity derived from the $C^{14}O_2$ was counted in an ionization chember, using a vibrating reed electrometer according to the method of Brownell and Lockhart.[7] During the first 24 hours, 22.2 and 13.4 per cent, respectively, of the total radioactivity administered were found in the urine (Table 1). The total amounts of radioactivity recovered from the urine during the 6-day collection

*We gratefully acknowledge the cooperation of Drs. R. Hertz and D. M. Bergenstal (Endocrinology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland) in this study.

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THE FATE OF INTRAVENOUSLY-ADMINISTERED C^{14}-21-PROGESTERONE IN THE HUMAN ORGANISM*

By

C. I. Lupu, E. J. Plotz, and M. E. Davis

Previous studies[1,2] have revealed that substantial amounts of radioactivity are excreted into the urine and the feces of pregnant and nonpregnant patients who had received intramuscular injections of isotopic progesterone labeled with carbon-14 at position 4. Negligible amounts of radioactivity were found in the fatty surface film of the skin, but none was found in the carbon dioxide of the expired air. The pattern of circulating curves for the concentration of radioactivity in the plasma suggested a rapid disappearance of the hormone and its metabolites from the blood. Relatively high concentrations of radioactivity were demonstrated in the fat, indicating storage of the hormone in the fat compartment.

In the following study, 28.3 μc of progesterone labeled with carbon-14 at position 21 were injected intravenously into a 35-year-old woman who was 10 weeks pregnant. A therapeutic abortion was performed 6 days after the injection because of severe sickle cell anemia. The male fetus weighed 33 g and measured 11.5 cm (CH) and 8.5 cm (CR). The weight of the placenta was 53.5 g.

Samples of the expired air were taken at various intervals during a period of 31 hours following the intravenous administration of the labeled progesterone. For each collection, the patient breathed into a polyethylene bag for 5 to 10 minutes. The bag was then transferred to a collection train in which the volume of expired air was measured and the carbon dioxide was trapped in aqueous sodium hydroxide. Addition of acid to the gas in a Roth combustion apparatus[3] released the carbon dioxide, the volume of which was measured and then collected in an ionization chamber. Radioactivity was estimated by means of a vibrating reed electrometer according to the method of Brownell and Lockhart.[4]

The radioactivity excreted into the urine and feces, as well as that found in maternal and fetal tissue, was estimated according to the methods described previously.[1,2] The blood of this patient was sampled at intervals throughout the experimental period and was fractionated into the free and conjugated steroid fractions according to the method of Bradlow and Gallagher:[5]

A 5-ml aliquot of blood plasma was added to 2.5 volumes of chloroform, alcohol, and acetone (1:1:1 V/V). After filtration and separation, the organic phase was evaporated to dryness. The resulting residue was partitioned between chloroform and water. After separation of the two phases and appropriate washing, the aqueous phase contained the con-

*We wish to acknowledge the valuable advice of Edward C. Tocus and the technical assistence of Reuben E. Chapman and Gerd Struver in these studies.

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Table 3

RADIOACTIVITY IN TOTAL PLASMA VOLUME IN THE UNCONJUGATED AND CONJUGATED STEROID FRACTION FOLLOWING THE INTRAVENOUS INJECTION OF C^{14}-21-PROGESTERONE

Radioactivity from	Percentage of injected radioactivity in total plasma volume*											
	minutes					hours						
	25	53	129	194	399	12	22	26	37	48	72	96
Unconjugated steroid fraction	1.83	0.79	0.23	0.46	0.10	0.28	-	0.05	0.05	0.10	0.07	0.0
Conjugated steroid fraction	5.75	5.22	4.34	4.19	2.57	1.79	0.80	1.63	0.76	0.60	0.51	0.26
Total	7.58	6.01	4.57	4.65	2.67	2.07	-	1.68	0.81	0.70	0.58	0.26

*Plasma volume equals 5 per cent of body weight.

after the intravenous injection of the tagged hormone and recovered 2.78 per cent of the administered dose in this sample. During the same time, not more than 3 per cent of the 'ioactivity was eliminated as carbon dioxide in the expired air. We have no reason to ume that appreciably larger amounts of radioactivity have been excreted by way of the bile during this time. Thus, we can account for no more than 10 per cent of the administered dose being eliminated by the kidneys, liver, and lungs within this short period. Nevertheless, only 4.65 per cent of the administered dose was present in the total plasma volume 194 minutes after injection. Therefore, it is logical to assume that a major part of the hormone and/or its metabolites diffuses rapidly from the blood plasma into the tissues, especially into the fat compartment from which it is returned more slowly to the circulation and subsequently excreted as metabolites by the kidneys and liver.

SUMMARY

Intravenously-injected C^{14}-21-progesterone is rapidly conjugated and removed from the circulating blood. About 18 per cent of the administered radioactivity is excreted as carbon dioxide in the expired air within a 31-hour period after the administration. Over a 9-day period, 22.62 per cent of the administered radioactivity was recovered from the urine, and over a 12-day period, 29.85 per cent in the feces. A relatively high concentration of radioactivity was still present in the maternal fat 6 days following the administration of the hormone, indicating the storage function of the fat compartment for the hormone and its metabolites.

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